Physiologically Based Pharmacokinetic Analyses of Simple Mixtures

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Exposure to multiple chemicals may cause significant alterations of tissue dose of the toxic moiety of one or more of the individual chemicals. The change in target tissue dose of a chemical present in simple mixtures can be predicted when the determinants of disposition of each chemical, and the mechanism of toxicokinetic interaction between chemicals are understood at a quantitative level. Determinants of disposition include physiological (e.g., breathing rates, cardiac output, tissue volumes, blood flow rates), biochemical (e.g., kinetic constants for metabolism and protein binding), and physicochemical factors (e.g., blood:air and tissue:blood partition coefficients). Mechanisms of toxicokinetic interactions refer to the manner in which coexposure alters these determinants of disposition as compared to exposure to the individual chemicals. Interactions between chemicals can be described quantitatively with physiologically based pharmacokinetic (PBPK) models, which integrate these mechanistic determinants and permit prediction of alterations in tissue dose for various exposure situations by computer simulation. PBPK modeling studies of binary chemical interactions conducted so far indicate that inhibitory rather than potentiating metabolic interactions are more likely to be observed during multiple chemical exposures. As PBPK models of representative binary, tertiary and quaternary mixtures are developed, it will become increasingly possible to draw reliable conclusions about the risk associated with human exposure to chemical mixtures. —Environ Health Perspect 102(Suppl 9):151–155 (1994)

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Introduction

Multichemical exposure is the rule rather than the exception in both the general and occupational environments. Simultaneous or sequential exposure to multiple chemicals may alter the toxicokinetics and/or toxicodynamics of one or all of them. This can lead to a quantitative alteration of the toxicity predicted based on the summation of the effects of the components. Toxicokinetic interactions occur when the tissue dose of the active chemical per unit of exposure is altered by co-exposure to other chemicals. Toxicodynamic interactions occur when tissue response to a unit tissue dose of the active chemical is altered by co-exposure to other chemicals. When interactions occur among components of a chemical mixture, the mechanistic basis of such interactions should be understood at a quantitative level to conduct risk assessment for the chemical mixture. The uncertainties arising from changes in the toxicokinetics of the components can be

addressed by developing physiologically based pharmacokinetic models of chemical mixtures which can be used for dose, route, and species extrapolations of target tissue concentrations of the toxic moieties. This paper presents a short overview of the basics of physiologically based pharmacokinetic modeling and some examples of its use in the mechanistic analyses of toxicokinetic interactions occurring in chemical mixtures.

Physiologically Based Pharmacokinetic Modeling

Physiologically based pharmacokinetic (PBPK) modeling is the process of developing mathematical descriptions of the uptake and disposition of chemicals in which the interrelationships among the critical biological determinants are described as realistically as possible. In the PBPK modeling approach, the body is divided into a number of tissue compartments (Figure 1), each of which is defined by appropriate volume, blood flow rates and solubility characteristics. The compartments may represent a single tissue or a grouping of tissues that have similar blood flow and solubility characteristics. In the PBPK models, the rate of tissue uptake of a chemical is described either as a blood flow limited uptake or limited by diffusion from blood into the tissue (I). For blood flow
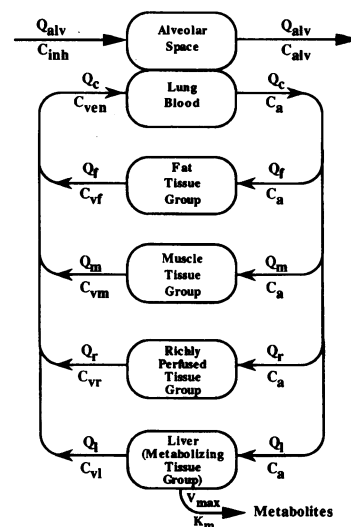

Figure 1. The structure of the physiologically based pharmacokinetic model for styrene. Q terms are air and blood flow rates; C terms are concentrations. These are indexed to individual tissue compartments: fat (f), muscle (m), richly perfused tissues (r) and liver (l). Effluent venous concentrations have a double lettered subscript. Q_{alv} and Q_t are alveolar ventilation and cardiac output. The subscripts inh, alv, art, and ven signify inhaled air, exhaled air, arterial blood, and venous blood, respectively. Kinetic constants for liver metabolism are V_{max} (maximum rate of metabolism) and K_m (binding affinity of the substrate with metabolizing enzyme). From Ramsey and Andersen (28), reproduced with permission of Academic Press.

limited uptake, the rate of change in the amount of a chemical in the tissue (dA_t/d_t) is described with a mass balance differential equation, which accounts for the roles of tissue blood flow rates (Q_t), arteriovenous concentration difference ($C_a - C_{vt}$), and tissue metabolism

$$dAt/d_t = Q_t (C_a - C_{vt}) - dA_{met}/d_t \quad [1]$$

Metabolism in individual tissues or tissue groups can be included by adding appropriate terms, to account for the amount lost by metabolism, which might be a first or second order process (e.g., glutathione conjugation), or a saturable process (e.g., cytochrome P450 mediated oxidation) as follows

$$dA_{met}/d_t = V_{max}C_{vt}/(K_m + C_{vt}) + K_f C_{vt} V_t \quad [2]$$

where

V_{max} = Maximum enzymatic reaction rate (mg/hr)
K_m = Michaelis constant for enzymatic reaction (mg/l)
K_f = First order rate constant (hr_{-1})
V_t = Volume of the tissue (l)

The total amount of the chemical in the tissue (A_t) then is calculated by integrating the mass balance differential equation (Equation 1). The tissue concentration of the chemical at any time is calculated by dividing the amount in the tissue by tissue volume.

Three types of parameters are required to develop PBPK models: physiological (e.g., alveolar ventilation rate, blood flow rate, tissue volumes, glomerular filtration rate), biochemical (e.g., V_{max}, K_m) and physicochemical (e.g., blood:air and tissue:blood partition coefficients). Partition coefficients of volatile organic chemicals can be determined by vial equilibration (2). Physiological parameters can be obtained from biomedical literature (3). Biochemical parameters related to metabolism and protein binding can be determined either in vitro or using noninvasive in vivo exposure techniques such as gas uptake and exhaled breath techniques for metabolic parameters (4). Once formulated by integrating the information on animal physiology, rate constants for kinetic processes and partition coefficients, the PBPK model can be used to simulate the kinetic behavior of a chemical in the test species for a variety of exposure scenarios.

This type of PBPK model has been developed for a number of individual chemicals (4). The principal application of this biologically and mechanistically based approach is in the prediction of target tissue dose of the toxic parent chemical or its reactive metabolite. Using the tissue dose of the toxic moiety of a chemical in risk assessment provides a better basis of relating to the observed toxic effects than the external exposure concentration (5). Because PBPK models facilitate the prediction of target tissue dose in people, they can help reduce the uncertainty associated with the conventional extrapolation procedures (6).

PBPK Modeling of Simple Mixtures

When animals and people are exposed to two chemicals there may or may not be an interaction between the chemicals. Toxic interactions result from the modulation of the toxicokinetics and/or toxicodynamics of one chemical by another. Toxicokinetic interactions involve modulation of the absorption, distribution, metabolism, and excretion of one chemical by another via alterations in the physicochemical, physiological, and biochemical parameters. PBPK modeling studies of chemical mixtures can significantly improve our ability to investigate mechanisms of toxic interactions in vivo in a quantitative manner, and can be used to conduct dose, route, and species extrapolations of the target tissue dose of the toxic moieties of the chemicals in the mixture.

For PBPK modeling of binary mixtures, the influence of one chemical on the other should be considered in terms of the alteration of critical biological determinants of disposition by the coexposure. Combined chemical exposures may affect (a) physicochemical parameters, (b) physiological parameters and (c) biochemical parameters, which necessarily determine the disposition of both chemicals, and their target tissue dose.

Physicochemical Parameters

During combined exposure scenarios, one chemical may alter the solubility characteristics of another chemical. For example, cyanide forms complexes with essential metals resulting in a change in their tissue concentrations and distribution pattern (7–10) due to changes in solubility and stability (11,12). Similarly, various dithiocarbamates form lipophilic complexes with inorganic lead, enhancing lead uptake across the blood-brain barrier, thus causing a greater accumulation in the lipid-rich brain compartments (13). However, the tissue:air and blood:air partition coefficients of certain volatile organic chemicals have been found to remain unaltered during combined exposures (14). These chemicals still interact by mechanisms that involve changes in the biochemical and physiological parameters.

Physiological Parameters

Physiological parameters include tissue volumes (V_t), breathing rates (Q_p), cardiac output (Q_c), blood flow rates (Q_t), glomerular filtration rate (GFR), etc. If one chemical in a simple mixture alters one of the physiological parameters, then that chemical can be expected to alter the disposition and target tissue dose of other components in the mixture (15). For example, repeated administration of phenobarbital causes enlargement of the liver (i.e., increases the model parameter V_l), and alters liver blood flow rates (in addition to altering the biochemical parameters). Ethanol causes alterations of hepatic blood flow rates (Q_l) and cadmium alters the GFR. Hydrogen sulfide and hydrogen cyanide at low exposure concentrations cause increases in Q_p, thus increasing the respiratory uptake (and therefore the toxicity) of other chemicals. For these observations to be incorporated into a physiological modeling framework to predict their effect on the disposition of other chemicals, dose-response information (e.g., Q_l, Q_p, V_l, GFR versus exposure concentration) is needed. With environmental chemicals, the most common single mechanism of interaction investigated in such detail appears to be the modulation of biochemical parameters (15).

Biochemical Parameters

Biochemical parameters include the rate constants of metabolism, and the affinities and capacities of protein binding. The effects of combined exposure to chemicals on their metabolism might be a result of their competition for enzymatic binding sites, or might be due to one chemical inducing the enzyme system implicated in the metabolism of the other.

Enzyme induction has been modeled by taking into account the altered V_{max} or K_f due to treatment with the inducer. Induction should not alter the K_m if the same isoform is induced. Andersen et al. (16) modeled the effect of prior administration of styrene (1000 ppm for 6 hr/day for 4 days) or phenobarbital (80 mg/kg/day for 4 days prior to styrene exposure) on the metabolism of styrene. Pretreatment with phenobarbital increased the V_{max} by a factor of six; styrene pre-exposures increased V_{max} by a factor of 2. These observations were consistent with a role for a high affin-
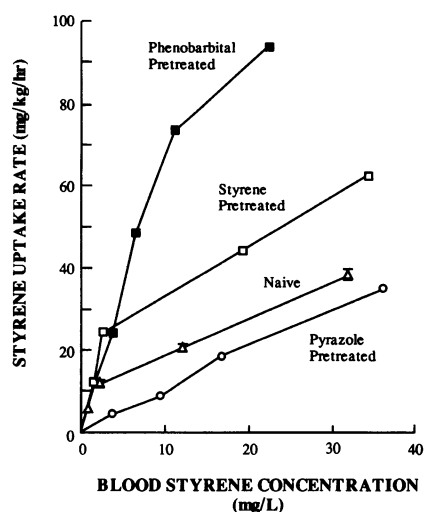
Figure 2. Relationship between the rate of uptake of styrene from inhaled air and the arterial blood concentration of styrene at the end of a 6-hr exposure. Pretreatments used were pyrazole, 320 mg/kg, 30 min before initiating exposure, and phenobarbital, 80 mg/kg/day on each of 4 days preceding styrene exposure. The styrene pretreatment was daily exposure to 1000 ppm for 6 hr on each of the 4 days before test exposure to the various concentrations. From Andersen et al. (16) with permission of the Academic Press.

Figure 3. Model simulations of the area under the blood 2,5-hexanedione concentration vs time curve for 6- and 12-hr inhalation exposures of up to 10,000 ppm n-hexane. The solid lines were generated with a PBPK model assuming competitive interactions among n-hexane, 2,5-hexanedione and methyl n-butyl ketone.

Figure 4. Model predictions (solid lines) and experimental observations (symbols) of carboxyhemoglobin levels during and following combined exposure to dichloromethane and isofluorene. In the PBPK model, the hepatic metabolism of both chemicals was described as a competitive inhibition process. From Clewell and Andersen (6); with permission of Princeton Scientific Publishing Inc.

ity enzyme in the metabolism of styrene, and indicated that the inducer pretreatments did not enhance styrene metabolism at low exposure concentrations (<200 ppm). A curve fitting routine was employed with the PBPK modeling approach to describe the kinetics of the enzyme induction process. This modeling aspect was based on a) basal V_{max} for styrene metabolism in naive rats, b) time lag for induction, c) incremental increase in Vmax, and d) a rate constant for the induction process. More recently, the influence of enzyme induction by several substances (phenobarbital, Mirex, and chlordecone) on the metabolism of $BrCCl_3$, and by ethanol on the metabolism of trichloroethylene have been described within a physiological modeling framework (17,18). At low exposure concentrations (i.e., when hepatic blood flow limits metabolism), the effect of enzyme induction will be negligible. In these instances, interactive effects could still be important if they involve metabolic inhibitions.

For elucidating the mechanism of metabolic inhibition, the mass balance differential equation of the metabolizing tissue (e.g., liver) has to be modified to accommodate various hypotheses (Table 1). The predictions of the PBPK model with each mechanistic description are then compared
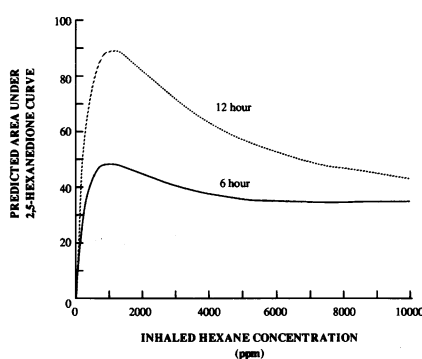
with experimental data on both chemicals obtained during coexposures, to test the validity of the hypotheses. Using the PBPK modeling approach, Andersen and Clewell (19) described the toxicokinetic interaction among n-hexane and its metabolites.

n-Hexane is metabolized by a saturable pathway yielding methyl n-butyl ketone (MnBK). MnBK is further metabolized by ω-1 oxidation to 2,5-hexanedione, the neurotoxic metabolite, and by α-oxidation and decarboxylation to pentanoic acid. Following a 6-hr exposure of F-344 rats to 500, 1000, 3000, and 10,000 ppm of n-hexane, the blood concentrations of n-hexane and MnBK increased linearly with increasing exposure concentrations, but 2,5-hexanedione (HD) showed anamolous behavior (20). The concentration of HD at the end of hexane exposure increased substantially from 500 to 1000 ppm, then remained fairly constant between 1000 and 3000 ppm but was lower for the 10000 ppm n-hexane exposure than for the lower exposure levels. At high hexane exposure concentrations, the blood level of HD immediately following hexane exposure was lower than the eventual peak, indicating that inhibition of HD
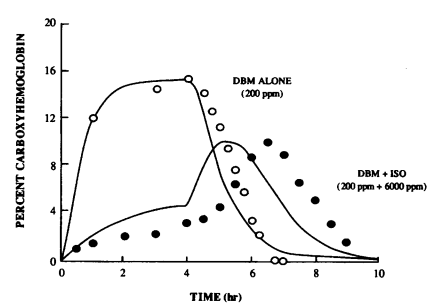
production during hexane exposure ceased with the termination of hexane exposure. In addition, the time at which the peak HD concentration occurred was immediately after exposure at lowest exposure concentrations, but not until 6 hr later for the highest exposure concentration. In modeling this complex kinetic behavior of HD resulting from hexane exposure, Andersen and Clewell (19) considered multiple competitive interactions among n-hexane, MnBK, and HD. This PBPK description consistent with the observed kinetics of n-hexane, MnBK and HD predicted a peak toxicity (corresponding to the increased AUC of HD) of n-hexane at 1000 ppm (Figure 3) and also suggested that both at higher concentrations and continuous exposures, n-hexane might actually be less toxic due to lower conversion to 2,5-HD than at intermediate concentrations and intermittent exposures. In the hexane model, HD and MnBK were also capable of inhibiting n-Hexane metabolism.

Inhibitory interactions similar to those occurring between hexane and MnBK, and HD can also occur during exposure to a binary mixture of the chemicals if the chemicals have similar properties. The

Table 1. Metabolic inhibition mechanisms and the corresponding equations used in the PB-PK models.

Inhibition type	Equation
None	$V_{max1} \times C_{v1} / (K_{m1} + C_{v1})$
Competitive	$V_{max1} \times C_{v1} / (K_{m1} \times (1 + C_{v2}/K_{i2,1}) + C_{v1})$
Uncompetitive	$V_{max1} \times C_{v1} / (K_{m1} + C_{v}1 \times (1 + C_{v2}/K_{i2,1})$
Noncompetitive	$V_{max1} \times C_{v1} / ((K_{m1} + C_{v1}) \times (1 + C_{v2}/K_{i2,1}))$

These equations are written for chemical 1 as substrate and chemical 2 as inhibitor.
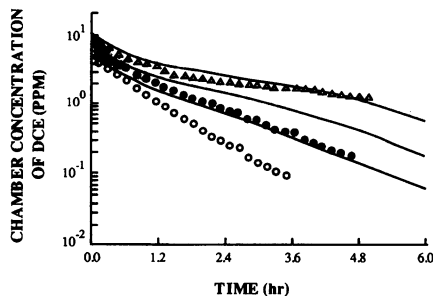
Figure 5. trans-1,2-Dichloroethylene uptake by groups of three F-344 rats placed in a closed, recirculated chamber with starting concentrations of 12, 8, or 5 ppm. Experimental data are shown as symbols and model simulations are presented as solid lines. The systematic discrepancy between the model and the data provided an indication that the simple description of metabolism in the model was inadequate for this chemical. From Clewell and Andersen (25); with permission of the National Academy of Sciences.
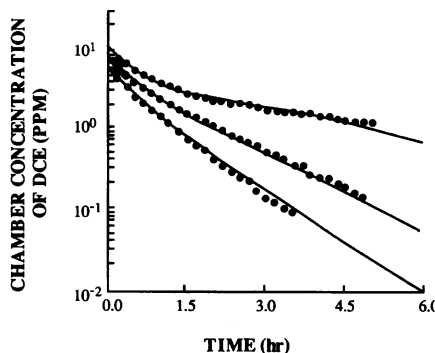

Figure 6. 1,2-Dichloroethylene uptake by groups of three F-344 rats placed in a closed, recirculated chamber with starting concentrations of 12, 8, or 5 ppm. Experimental data are shown as symbols and model simulation is presented as solid lines. In this case, the model description accounted for enzyme inactivation by reactive metabolites assumed to be produced during the metabolism of trans-dichloroethylene. From Clewell and Andersen (25) with permission of the National Academy of Sciences.

inhibitory chemical (equivalent to hexane) needs to have a low partition coefficient and be rapidly eliminated by exhalation at cessation of the co-exposure period. The metabolized component (equivalent to MnBK) should have a greater solubility and persist in the body after the exposure is halted. To facilitate the study, metabolite (equivalent to HD) should readily be measurable. Clewell and Andersen (6) devised

a mixture of isoflurane (ISO) and dibromomethane (DBM). DBM, a tissue-soluble vapor, is metabolized to carbon monoxide. During exposure ISO inhibits DBM conversion to CO. After exposure ISO is eliminated more rapidly than DBM and CO production is enhanced. The complex behavior of carboxyhemoglobin, like that of HD, occurs due to differential blood:air partition coefficients of the competing substrates (Figure 4).

Inhibitory metabolic interactions between trichloroethylene and dichloroethylene, benzene and toluene, and m-xylene and toluene have also been described with a physiological modeling approach (21–23). In these studies, the metabolic rate constants for each chemical were first determined by conducting gas uptake studies with individual chemicals, and then the metabolic inhibition constants were determined by conducting another series of gas uptake studies with both chemicals. The binary chemical gas uptake data indicating altered uptake of both chemicals during coexposures were analyzed with a PBPK model to test various hypotheses of inhibitory interaction (e.g., competitive, noncompetitive, uncompetitive). These studies considered the metabolic rate constants to be time-invariant. There are instances where such a description may not be sufficient, especially where inactivation of metabolizing enzymes occurs during the exposure.

Andersen et al. (24) found that the decline in the gas uptake chamber concentration of both cis- and trans- 1,2-dichloroethylene could not be described with time-invariant metabolic constants (Figure 5). This indicated that the maximum rate of metabolism was decreasing during exposure to these compounds. The gas uptake data was successfully described by a PBPK model in which the rate of enzyme inactivation was proportional to a second order rate constant (K_d) times the square of the instantaneous rate of metabolism (Figure 6). The square dependence on instantaneous metabolic rate indicated an interaction between a reactive metabolite and the enzyme-substrate complex in the rate limiting step for enzyme inactivation (25,26). Of the two chloroethylenes, the trans isomer is a much better suicide inhibitor than the cis isomer (K_d: 400 vs 1.2). Inhibition

by trans-1,2-dichloroethylene occurs at low exposure levels (5 ppm), and may be significant in various exposure situations.

Future Directions

Binary chemical mixtures are a great simplification of the real world situations. The toxicokinetics and toxicodynamics of two interacting chemicals might further be altered by other components in more complex mixtures. With multichemical mixtures, some components may act independently, neither interfering with nor being modified by other chemicals, whereas others might interfere with and modify the toxicity of other chemicals. With environmental chemicals, toxic interactions mainly appear to involve alteration of biochemical parameters (15). If metabolic inhibitory interaction occurs among the components of a multichemical mixture, the modeling of such a phenomenon can be accomplished by approaches similar to those utilized for binary chemical mixtures (27).

Another area that deserves more consideration is the role of multiple forms of cytochrome P450 each of which may metabolize a given chemical with distinct affinity and capacity. PBPK modeling of multiple chemicals metabolized to varying extents by several isoenzymes will be more complicated with various mixed type inhibitions, rendering the discrimination between mechanistic descriptions difficult.

In summary, physiologically based modeling approaches facilitate predictions of change in the target tissue concentrations of toxic moiety of chemicals present in simple mixtures, when the mechanisms of disposition and interaction are understood at a quantitative level. The PBPK analyses of simple mixtures conducted to date indicate that the effects of enzyme inactivators, such as trans-1,2-dichloroethylene, are likely to be observed in occupational and perhaps in certain environmental exposure situations. On the contrary, the interactive effects of enzyme inducers such as ethanol and styrene will be important only at much higher exposure concentrations. As PBPK models of representative binary, tertiary and quaternary mixtures are developed, it will become increasingly possible to draw reliable conclusions about the risk associated with human exposures to chemical mixtures.

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